Analyses of the August 2, 2004 Performance Evaluation Results for *M. tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention

Overall Summary of Results

***M.tb* positive and negative samples:**

			3 Positive Samples TB04-08-1 TB04-08-3 TB04-08-5	2 Negative Samples TB04-08-2 TB04-08-4	
Method	**Total # of laboratories**	**Total # of results**	**False-negative results**	**False-positive results**	**Overall Performance**
Gen-Probe MTD	64	320	None	5/128 (3.9%)	98.4%
Roche Amplicor	16	80	None	None	100.0%
In-house/Other	6	30	None	None	100.0%

New Findings

- In this shipment, sample TB04-08-2, contained a high concentration (3.0×10^6 theoretical cells/ml) of *M. celatum*. Of all participants, 84.9% (73/86) reported the interpretation as negative and 9.3% (8/86) reported the interpretation as inhibited. The interpretations reported as inhibited could have been due to the high concentration of non-tuberculous mycobacteria in the sample. *M. celatum* is still reported to potentially cross-react in the Gen-Probe® test (8); five of eighty-six (5.8%) laboratories using the Gen-Probe® test incorrectly reported the interpretation as positive.

- Results for sample TB04-08-4, containing 3.0×10^5 theoretical cells/ml of *M. scrofulaceum*, were reported as inhibited by 31.3% (5/16) of laboratories using the Roche Amplicor® method. Again, this could have been due to the high concentration of non-tuberculous mycobacteria in the sample interfering with the nucleic acid amplification.

- It is a concern that 7.1% (6/85) of responding laboratories reported that unidirectional workflow is not used. This has decreased from 11.4% (10/88) from the January 2004 shipment.

Findings of note that also have been reported previously

- Fifty-eight of eighty-six (67.4%) participants performed inhibition testing on *M.tb* NAA-negative specimens. This was an increase compared with the January 2004 shipment in which 58.3% (49/84) performed inhibition testing on negative specimens. The current *M.tb* NAA CDC testing algorithm includes recommendations for inhibition testing on negative specimens (1).

- Of the laboratories that received processed specimens for testing, 46.9% (30/64) indicated that they inquire about the sample submission buffer. This proportion has remained similar over the last several shipments.

Introduction

This report is an analysis of laboratory test results reported to the Centers for Disease Control and Prevention (CDC) by participant laboratories for the samples containing *M. tuberculosis* or non-tuberculous mycobacteria shipped in August 2004. Responses were received from 86 of 91 (94.5%) laboratories participating in this shipment. The *M.tb* NAA Performance Evaluation Program provides laboratories with a tool for external quality assessment. To maintain participant confidentiality, the CDC analyzes only participant data from which all laboratory identifiers have been removed by the contractor, Wisconsin State Laboratory of Hygiene (WSLH).

Challenge Samples

Participant laboratories received five individual samples. Participants were requested to test the samples without the decontamination and concentration procedures routinely performed on respiratory specimens prior to *M.tb* NAA testing. The specimen decontamination/concentration preparation steps for *M.tb* NAA testing were eliminated to allow this program to specifically assess *M.tb* NAA testing procedures (2,6).

Experiments were performed to document sample viability and test reactivity. Due to specific concerns of cross-contamination between *M.tb* NAA-positive and *M.tb* NAA-negative test samples, the negative samples were produced in a separate area. Additionally, 10% of both positive and negative samples were randomly selected and tested by the contractor to validate *M.tb* NAA results. The samples were also tested by five reference laboratories before shipping.

Results

Figure 1 shows the laboratory classification represented by 83 participants. Participants consisted of 36 hospitals, 36 health departments, 10 independents, and 1 other type of laboratory.

Figure 2 provides the distribution of the volume of specimens tested with *M.tb* NAA by participating laboratories during the 3 months prior to reporting results.

Figure 3 provides a breakdown of the *M.tb* NAA test procedures reported by the participating laboratories. Participants were asked to check all test methods used. All of the participants (6/6) reporting the use of In-house *M.tb* NAA test procedures used methods based on polymerase chain reaction (PCR). Although the CDC does not recommend the use of non-FDA cleared *M.tb* NAA test procedures (3,5), laboratories using In-house methods are encouraged to participate in this evaluation program to assess performance (2).

Figure 4 lists the biosafety levels reported by participant laboratories. All laboratories should routinely consult the CDC/NIH manual, Biosafety in Microbiological and Biomedical Laboratories (4^{th} edition), for recommendations and for determining their correct biosafety level.

Participants were also asked to provide information on specific quality control practices related to the prevention of cross-contamination and subsequent false positives with NAA testing.

Figure 5 provides the participant laboratory responses to a question about whether the biological safety cabinet (BSC) used for *M.tb* NAA testing is used for other purposes. One concern is that 13% (11/86) of participant laboratories indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 28% (24/86) of participants that indicated "Other" uses for the *M.tb* NAA testing BSC, 13 performed *M.tb* testing procedures or culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 11 performed mycology, and two performed other microbiology or clinical specimen work. Two laboratories reported using the same BSC for bioterrorism-related work. Laboratories should be aware of recommendations (4) to perform specimen processing and NAA testing in separate work areas with separate equipment to avoid contamination problems.

Figure 6 provides participant responses to a question on the use of uni-directional workflow for *M.tb* NAA testing. In addition to recommendations (4) that emphasize considerations of laboratory design for NAA testing, both manufacturers (Roche Amplicor® and Gen-Probe® MTD) recommend the use of unidirectional workflow. It is a concern that 7.1% (6/85) of responding laboratories reported that unidirectional workflow is not being used.

Separate figures and tables are provided to show either the qualitative or quantitative results reported for each sample by the participant laboratories. Quantitative results for the In-house methods could not be presented in a consistent format since participants used a variety of detection systems and test interpretation criteria. The Roche Amplicor® test has interpretive criteria for quantitative results that reflect some probability that the sample is positive but is below the recommended threshold for positivity. The result form and this report use the term "equivocal" for Roche Amplicor®, to reflect the manufacturer's recommendation for reporting indeterminate quantitative test results.

Figure 7 provides a summary of the participant qualitative results reported for all five samples by test method. The aggregate participant qualitative results are indicated for the 3 positive and 2 negative samples. The combined analytical sensitivity of all methods was 100% (258/258) for the TB04-08-1 (3.0×10^6 theoretical cells/ml), TB04-08-3 (3.0×10^5 theoretical cells/ml) and TB04-08-5 (3.0×10^5 theoretical cells/ml). The combined analytical specificity of all methods was 97.1% (167/172) for the 2 negative samples TB04-08-2 (3.0×10^6 theoretical cells/ml of *M. celatum*) and TB04-08-4 (3.0×10^5 theoretical cells/ml of *M. scrofulaceum*): 96.1% (123/128) specificity for Gen-Probe®; 100% (32/32) specificity for Roche Amplicor®; 100% (12/12) specificity for In-house methods. Interpretations reported as inhibited were considered correct for samples TB04-08-2 and TB04-08-4. The inhibition observed in these samples was confirmed by WSLH laboratory.

Figure 8 is graphical representation of the quantitative results reported for each sample by participant laboratories using the Gen-Probe® MTD test. The indention in each box-plot indicates the median value. The shaded area within the box represents the results between the 25^{th} percentile and 75^{th} percentile of the data. The bracketed areas designate either 1.5 times the interquartile range of the data or the most extreme data point on either side of the median, whichever is the least distance from the median. Each value reported which was outside these ranges is signified by one of the solid lines drawn outside the brackets. For the positive samples, TB04-08-1, TB04-08-3, and TB04-08-5 the median values of all data were 2,987,975; 3,013,968 and 3,012,013 relative light units (RLU), respectively. The median value for the negative sample containing *M. celatum,* TB04-08-2, was 8,482 relative light units (RLU). The median and range

of values reported for this sample was higher than that of a typical negative sample. Most of the lines shown as outliers on the graph were values from 12.5% (8/64) of laboratories reporting either false positive or inhibited interpretations. However, there were three outlying values which laboratories reported as negative. Overall the distribution of values reflected an indication of cross-reactivity. The median value for *M. scrofulaceum,* TB04-08-4, was 3,281 relative light units (RLU). The median and range of values overall was similar to a typical negative sample.

Figure 9 is a graphical representation of all quantitative results reported for each sample by participant laboratories using the Roche Amplicor® test. The solid line through each set of data represents the median value for each sample. The shaded band represents the equivocal range. The median value for positive samples, TB04-08-1, TB04-08-3, and TB04-08-5 were 3.013 (A_{450}), 3.298 (A_{450}), and 3.045 (A_{450}) respectively. The median values for the samples containing *M. celatum,* TB04-08-2, and *M. scrofulaceum*, TB04-08-4, were 0.050 (A_{450}), and 0.045 (A_{450}) respectively.

Sample TB04-08-2 contained a theoretical concentration of 3.0×10^6 cells/ml of *Mycobacterium celatum*, representative of a smear positive specimen. Table 2 shows that 5 (7.8%) of Gen-Probe users reported this specimen as positive, and 3 (4.7%) reported inhibition. For Roche users, none reported the specimen as positive, but 5 (31.3%) reported inhibition. The 6 laboratories using in-house methods reported the specimen as negative. *M. celatum* is known to be a cause of false-positive results with the Gen-Probe system, and fortunately is rarely encountered in clinical specimens (8,10). To optimize the specificity of the assay it is important to strictly adhere to the prescribed incubation temperatures and times for hybridization and selection steps. Temperatures should be monitored with each run using calibrated thermometers. Laboratories that obtained positive results for this sample should review the assay parameters and ensure that they are being followed. Cross contamination could also account for a false positive result, therefore, review of workflow and technique is suggested.

Large concentrations of non-tuberculous mycobacteria (MOTT) (e.g. $>10^5$ cells/ml) may inhibit the detection of small concentrations of the *M. tuberculosis* complex in nucleic acid amplification tests (8,9). The primers in the assays are genus-specific, and excessive numbers of MOTT may cause false negative results due to competitive amplification. Of 12 laboratories using the Roche system that reported testing for inhibition, 7 did not detect inhibition. The laboratories may not have done inhibition testing on these particular samples.

Sample TB04-08-4 contained a theoretical concentration of 3×10^5 cells/ml of *Mycobacterium scrofulaceum.* Table 4 shows that all Gen-Probe users reported the sample as negative. Five (31.3%) Roche users reported inhibition and 11 (68.6%) reported the sample as negative. The 6 laboratories using in-house methods reported the sample as negative. The same 7 laboratories that did not detect inhibition in TB04-08-2 also did not detect inhibition in this sample.

Overall, laboratories did well on this sample shipment. Sensitivity was 100.0% for samples containing *M. tuberculosis.* The nature of the negative samples demonstrated a couple of factors that can lead to false positive results or inhibited results with non-tuberculous mycobacteria. Sample TB04-08-2 contained high concentrations of *M. celatum* which can cross-react with tuberculosis in nucleic amplification tests (8,10). This sample and sample number TB04-08-4 (containing *M. scrofulaceum*) contained high concentrations of organism which could cause

inhibition by competing for primers in nucleic acid amplification tests. Laboratories should be aware of these potential issues.

We acknowledge the help of WSLH staff, Dr. David Warshauer, Mr. Phil Wand and Sue Legois, in contributing to interpretations of this data.

References

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10. Tjhie, JHT, van Belle AF, Dessens-Kroon M, and van Soolingen, D. Misidentification and diagnostic delay caused by a false-positive amplified *Mycobacterium tuberculosis* direct test in an immunocompetent patient with a *Mycobacterium celatum* infection. J. Clin. Microbiol. 2001; 39:2311-2312.

Figure 1. Primary Classification of Participating Laboratories

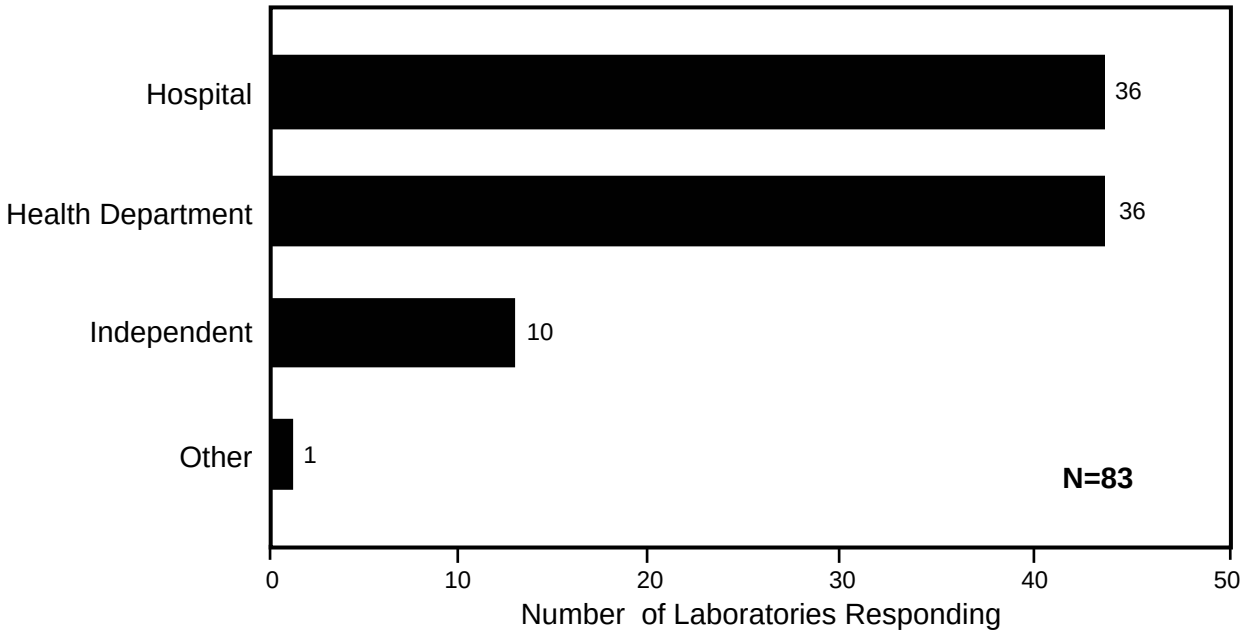
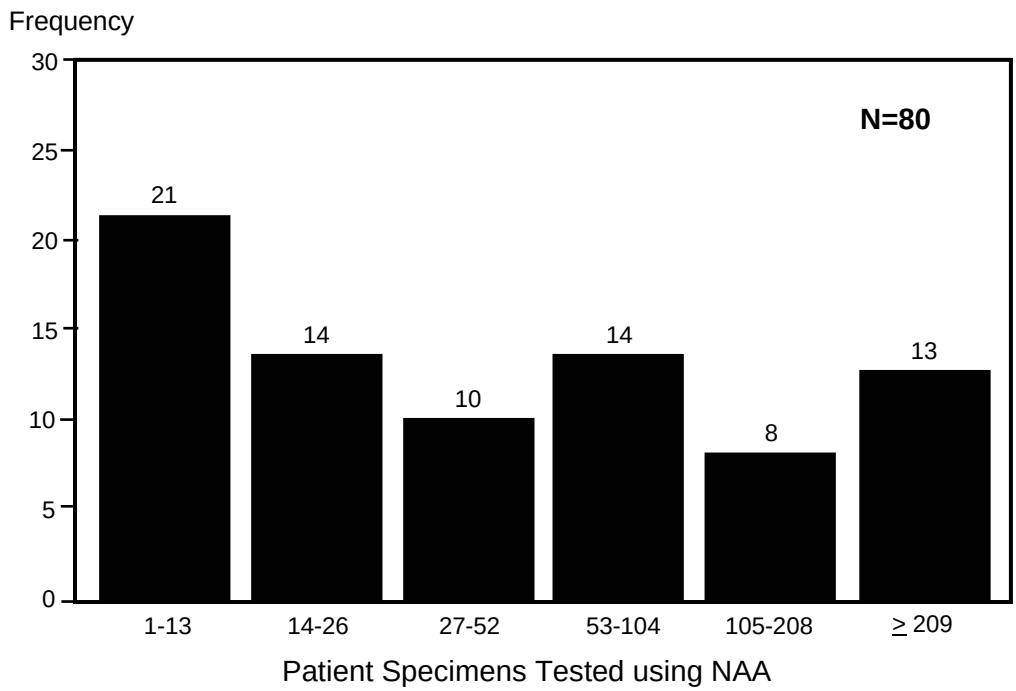


Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.*



*See explanation in the analysis.

Figure 3. Amplification Procedure Used for Direct Detection of *M.tb*

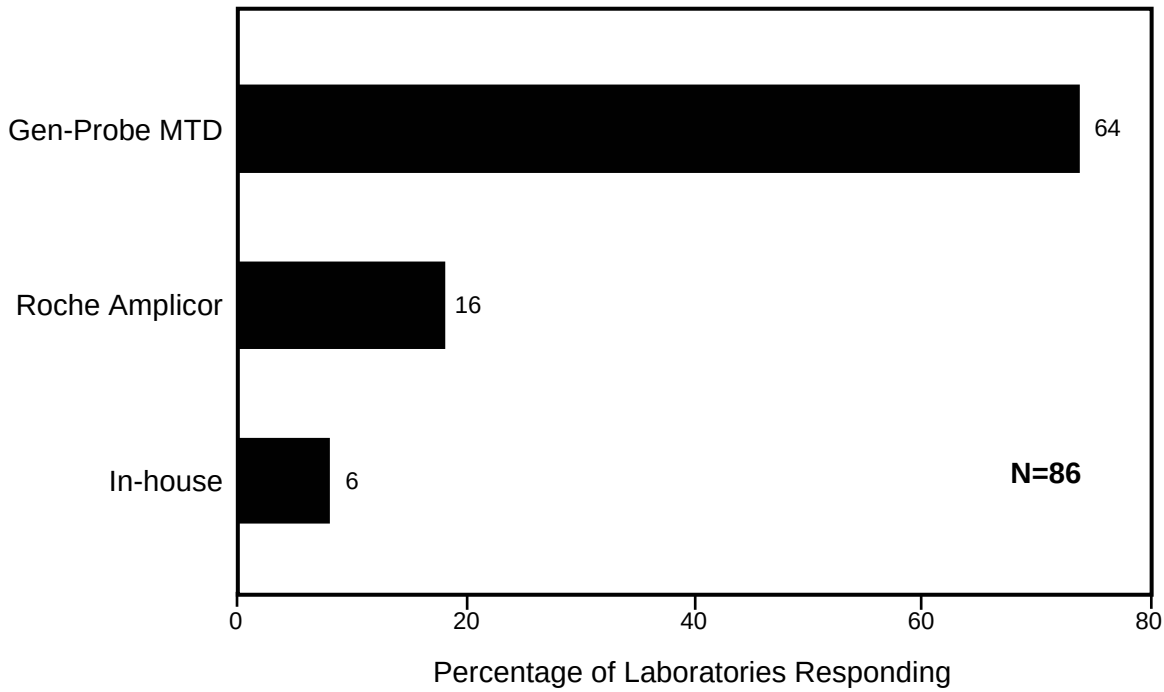


Figure 4. Biosafety Levels of Participant Laboratories

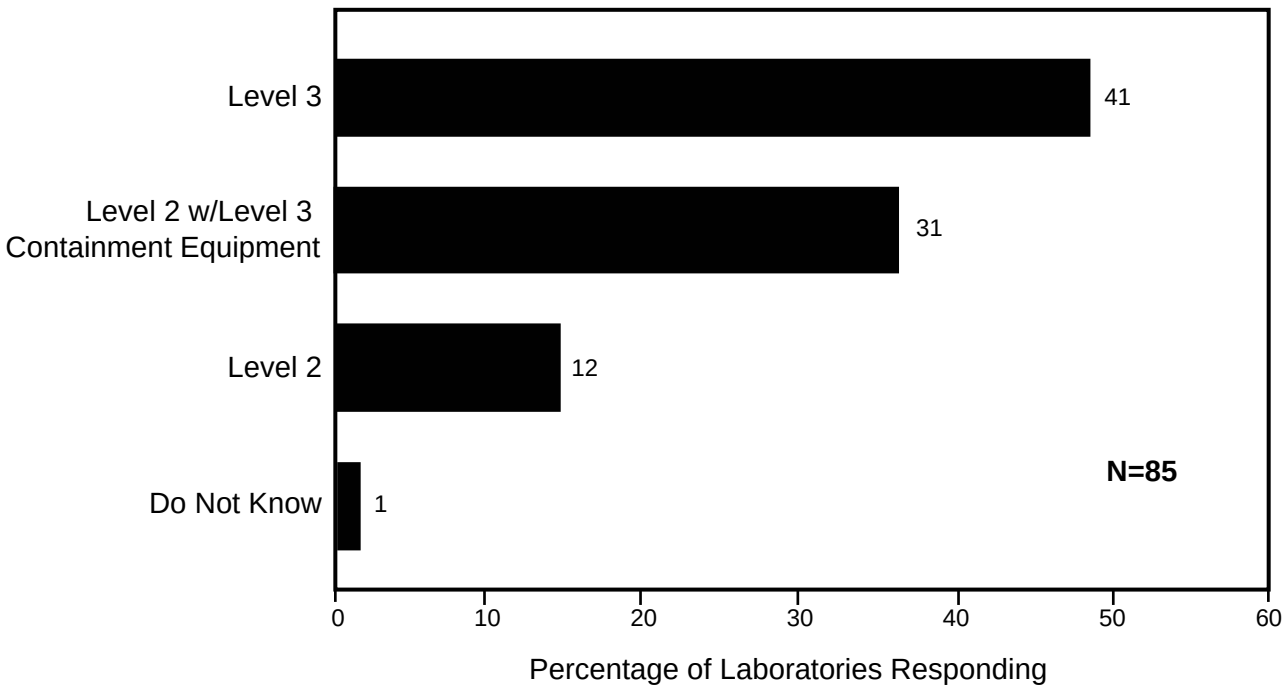


Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

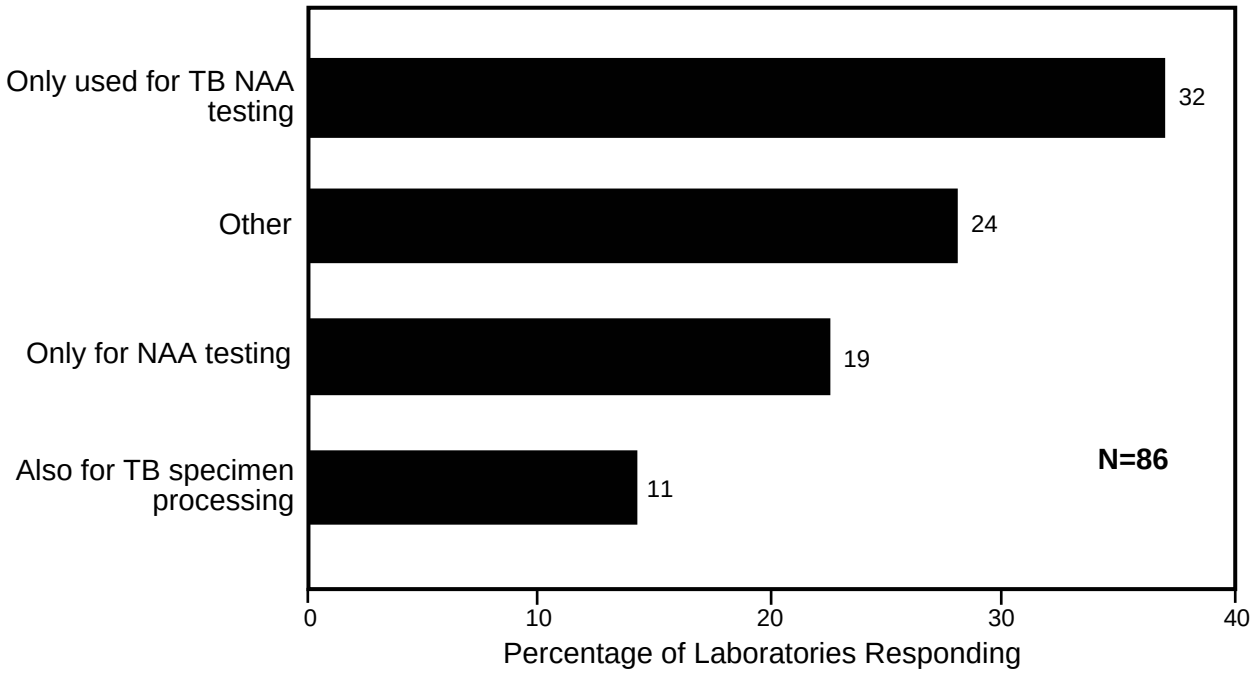


Figure 6. Use of Uni-directional Workflow by Participating Laboratories

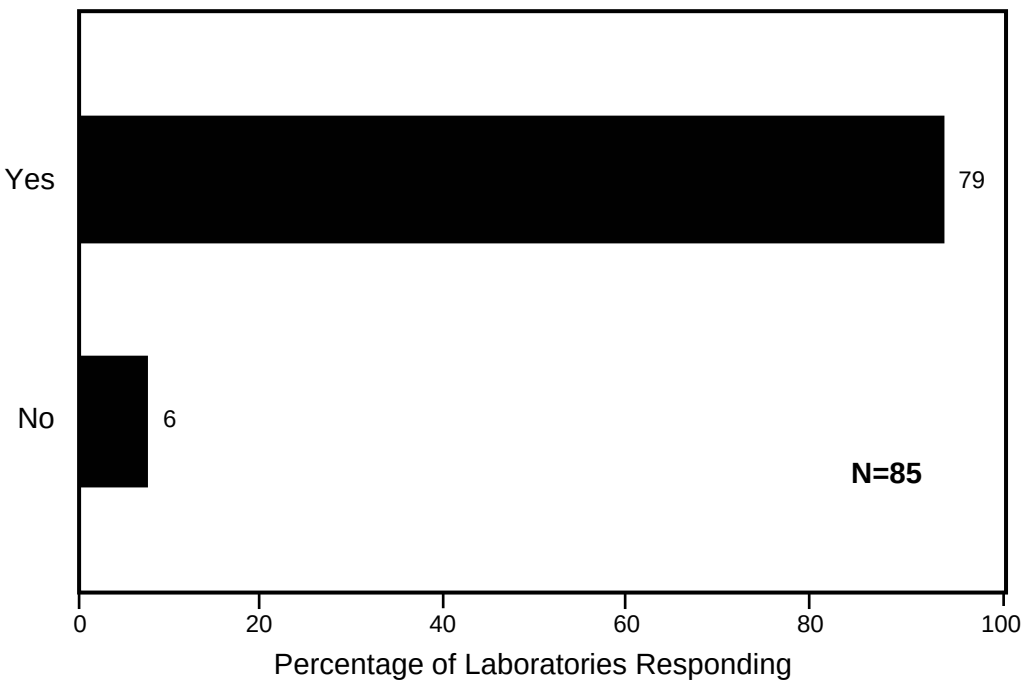


Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

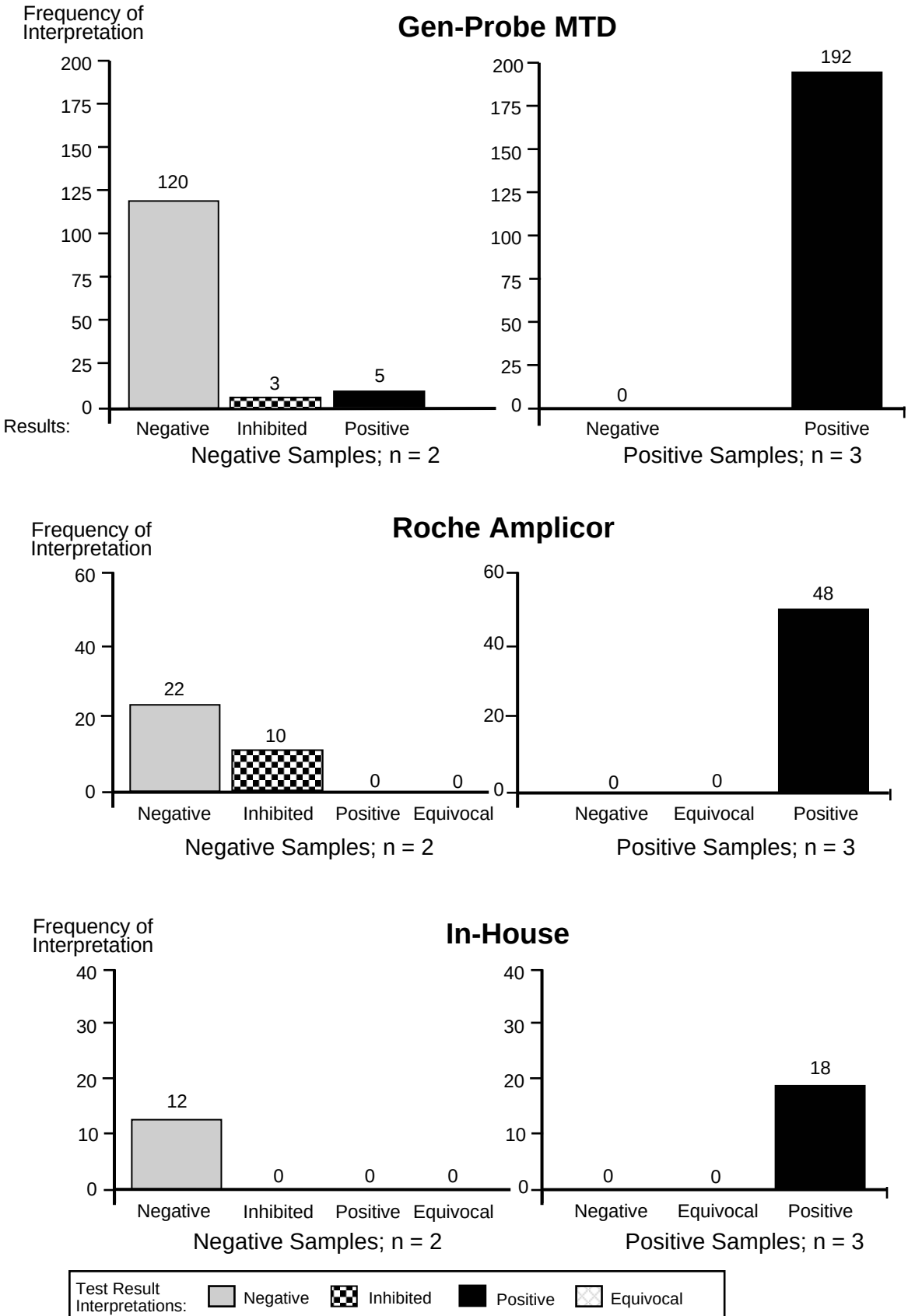
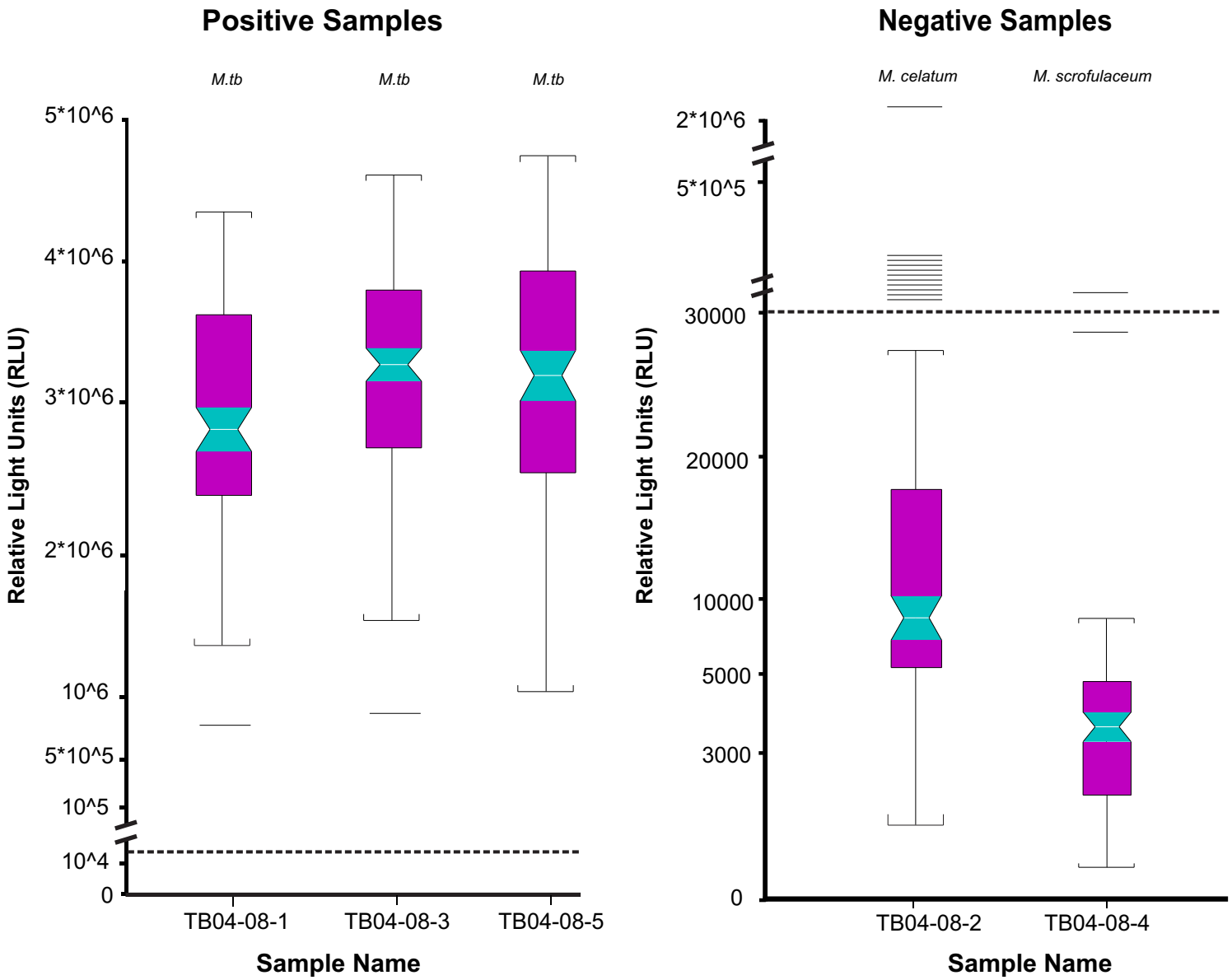
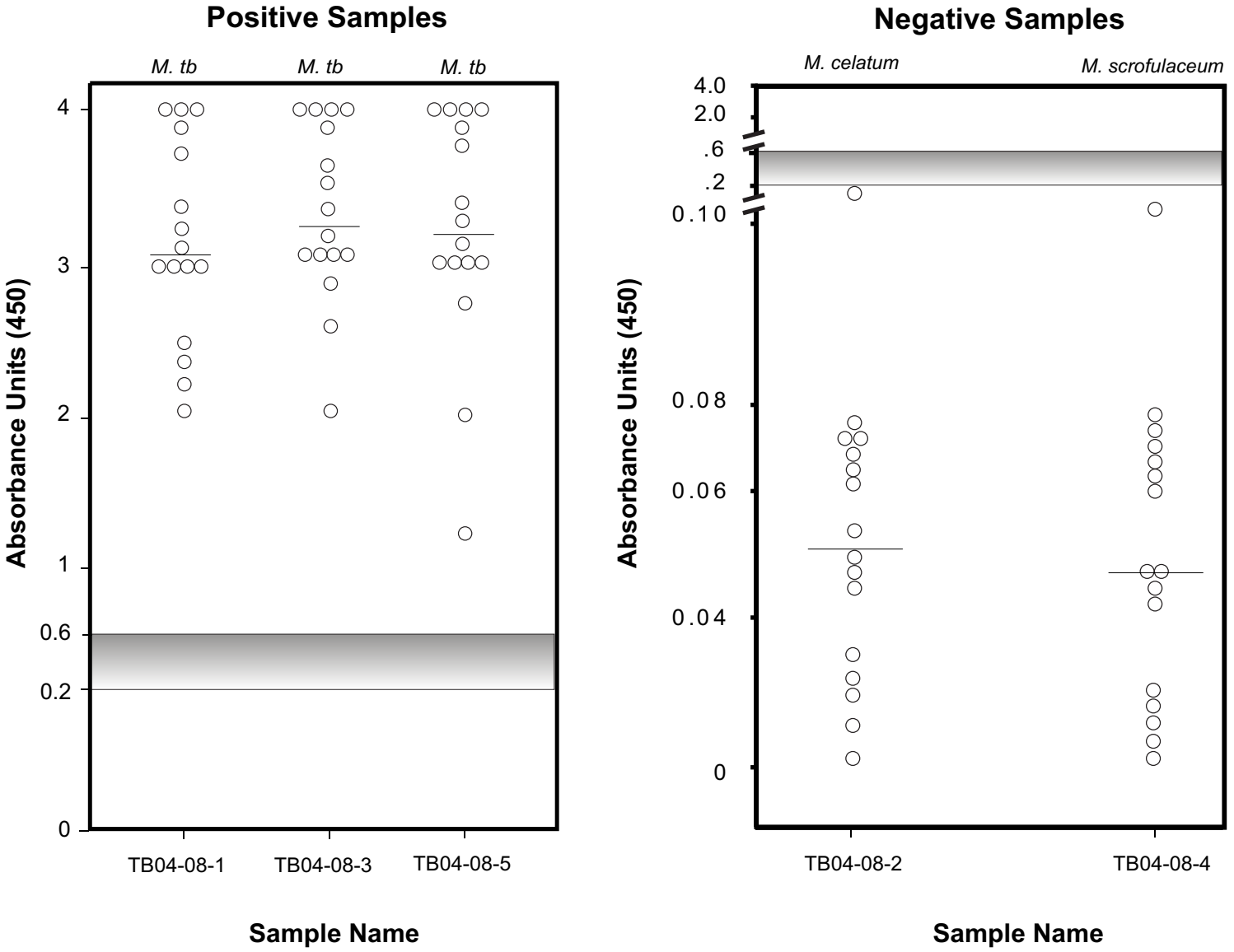


Figure 8. Quantitative Results for GenProbe® MTD



Note: Dashed line (**---**) represents cut-off between positive and negative values (30,000 RLUs).

Figure 9. Quantitative Results for Roche Amplicor®



Note: Shaded areas represent equivocal range.

The following tables summarize qualitative results reported by participant laboratories for the August 2004 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB04-08-1 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition Not applicable	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	64	64	100.0		n/a	n/a	0	0.0
In-house	6	6	100.0		0	0.0	0	0.0
Roche	16	16	100.0		0	0.0	0	0.0
All methods	86	86	100.0		0	0.0	0	0.0

Table 2. Sample TB04-08-2 contained *Mycobacterium celatum*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	64	5	7.8	3	4.7	n/a	n/a	56	87.5
In-house	6	0	0.0	0	0.0	0	0.0	6	100.0
Roche	16	0	0.0	5	31.3	0	0.0	11	68.8
All methods	86	5	5.8	8	9.3	0	0.0	73	84.9

Table 3. Sample TB04-08-3 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition Not applicable	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	64	64	100.0		n/a	n/a	0	0.0
In-house	6	6	100.0		0	0.0	0	0.0
Roche	16	16	100.0		0	0.0	0	0.0
All methods	86	64	100.0		0	0.0	0	0.0

Table 4. Sample TB04-08-4 contained *Mycobacterium scrofulaceum*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	64	0	0.0	0	0.0	n/a	n/a	64	100.0
In-house	6	0	0.0	0	0.0	0	0.0	6	100.0
Roche	16	0	0.0	5	31.3	0	0.0	11	68.8
All methods	86	0	0.0	5	5.8	0	0.0	81	94.2

Table 5. Sample TB04-08-5 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition Not applicable	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	64	64	100.0		n/a	n/a	0	0.0
In-house	6	6	100.0		0	0.0	0	0.0
Roche	16	16	100.0		0	0.0	0	0.0
All methods	86	86	100.0		0	0.0	0	0.0